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The role of anaerobic bacteria in the cystic fibrosis airway

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Abstract (word count, 192)

Purpose of review

Anaerobic bacteria are normal commensals but are also considered opportunistic pathogens and have been identified as persistent members of the lower airway community in people with cystic fibrosis (CF) of all ages and stages of disease. Currently, the role of anaerobic bacteria in CF lower airway disease is not well understood. Therefore, this review describes the recent studies relating to the potential pathophysiological role(s) of anaerobes within the CF lungs.

Recent findings

The most frequently identified anaerobic bacteria in the lower airways are common to both CF and healthy lungs. Studies have shown that in CF the relative abundance of anaerobes fluctuates in the lower airways with reduced lung function and increased inflammation associated with a decreased anaerobic load. However, anaerobes found within the lower airways also produce virulence factors, may cause a host inflammatory response and interact synergistically with recognised pathogens.

Summary

Anaerobic bacteria are potentially members of the airway microbiota in health but could also contribute to the pathogenesis of lower airway disease in CF via both direct and indirect mechanisms. A personalised treatment strategy that maintains a normal microbial community may be possible in the future.

Key words: anaerobes, microbial community, respiratory infection.

Introduction

Cystic fibrosis (CF) is the most common lethal inherited condition affecting the Caucasian population. Clinical characteristics are caused by the absence or reduction of cystic fibrosis transmembrane conductance regulator (CFTR) protein function, which perturbs ion channels at the apical membrane of epithelial cells and results in a multisystem disease. Disease of the lower airways is considered the most important manifestation as it is the cause of morbidity and mortality in a large percentage of people [1].

Lower airway disease is secondary to chronic bacterial infection and a dysregulated host inflammatory response that is dominated by neutrophils [2]. Currently, a limited range of bacteria which cause lower airway infection, including *Staphylococcus aureus*, *Haemophilus influenzae, Pseudomonas aeruginosa* and *Burkholderia cepacia* complex, have been correlated with worse outcomes of people with CF (Figure 1) [3]; therefore, they are recognised as CF pathogens and are usually targeted by antibiotic treatment regimens [4]. These pathogens are routinely detected using aerobic culture-based methods [5]; however, such methods fail to culture obligate anaerobic bacteria whilst non-target facultative anaerobic microorganisms isolated are often considered “oropharyngeal flora”.

During the past decade, unexpected bacterial community richness (number of species) within the CF lower airways has been demonstrated [6-12]. Furthermore, anoxic niches are known to exist within CF sputum, which supports the survival of anaerobic bacteria in the lower airways [13,14]. Many anaerobic species detected in CF respiratory samples are also present in the microbiota of the oral cavity and in a recent study, periodontal-associated

anaerobic bacteria were found in plaque and sputum samples of some people with CF [15*]. Therefore, detection of oral-associated bacteria in the lungs may be attributed to migration from the oral cavity to the lower airways [16-18].

Despite the accumulating knowledge of the range and abundance of bacteria in the diverse, dynamic and spatially heterogeneous lower airway communities, the contribution of obligate and non-target facultative anaerobic bacteria (henceforth referred to collectively as “anaerobic bacteria”) to the pathogenesis of CF lower airway disease is currently not well understood. In fact, there remains much deliberation about whether anaerobic bacteria are pathogenic in the CF lower airways, commensals with negligible clinical impact or members of a “normal” airway microbiota. This review focuses on the most recent knowledge pertaining to the potential role of anaerobes detected within the polymicrobial communities of the CF lungs.

Potential pathophysiological role of anaerobic bacteria in chronic lower airway infection

Ecological analyses generally describe that a reduced bacterial diversity, including decreased richness of anaerobes, leads to dominance of a recognised pathogen and is associated with antibiotic use, increasing age and poorer lung function [19-22]. Furthermore, it has been hypothesied that certain anaerobic bacteria potentially form part of a normal airway microbiota; the most frequently identified anaerobic genera (*Prevotella,* 83%; *Veillonella*, 54%) in CF sputum were also commonly detected in induced sputum of healthy controls [23*].

Variation in the abundance of anaerobic bacteria: impact on clinical status

It was recently shown that worse lung function (measured by lung clearance index) and increased inflammation (measured by blood C-Reactive Protein) were associated with a lower total load of anaerobic bacteria in clinically stable people with CF (Figure 2) [23*]. This suggests that a more diverse lung microbiota is potentially associated with health whilst a lower load of anaerobic bacteria may reflect microbiota disruption and disease progression (Figure 2) [23*]. However, some anaerobes in low abundance can have an adverse impact on disease progression, as has been demonstrated in periodontal disease [24].

Conflicting reports of variability in microbial community composition at the onset of an exacerbation exist with some studies reporting within-patient fluctuation whilst others finding little change compared with other time-points [9,25,26]. Recently there has been interest pertaining to microbial physiology within the polymicrobial community of the CF lower airways and using a novel microbial culture system, the physiochemical properties of the microbiota in sputum were investigated [27**]. Increased gas bubbles and reduced pH in glass capillary tubes, indicators of fermentative metabolism, at the onset of a pulmonary exacerbation coincided with a high abundance of fermentative anaerobes in a single patient with CF [27**]. Treatment with antibiotics raised the pH and reduced gas production and the abundance of anaerobes [27**].

It was also proposed that fluctuations in the abundance of specific anaerobic bacterial genera or species within the microbial community could potentially be utilised as a surrogate marker to indicate clinical status [20,28,29*]. This

includes the obligate anaerobes, *Porphyromonas* and *Prevotella* species, which when present in high relative abundance were associated with better lung function (measured by FEV_1) [28]. In contrast, an increase in the abundance of the facultative anaerobe, *Streptococcus anginosus* group, correlated with poorer lung function [28]. Furthermore, detection of *Streptococcus pyogenes* in high numbers was associated with acute pulmonary exacerbations in a small number of adults (n=15) with CF [30] whilst *Veillonella* and *Prevotella* species were predicted to represent potential surrogate markers for the onset of a pulmonary exacerbation in another recent study [29*].

Genetic adaptation and antimicrobial resistance

It is evident that anaerobic bacteria persist in the CF lower airway community, even under continuous antibiotic pressure targeting recognised pathogens [31]. In order to become established members of a bacterial community, bacteria may undergo genetic adaptation to a specific niche. For example, various clonal lineages of *P. aeruginosa* are known to accumulate mutations in genes that enable adaptation of the bacterium to the dynamic CF airways including mutations that increase resistance to antibiotics or affect virulence traits (described as “pathoadaptation”) [32]. Anaerobes from the CF lower airways are also more resistant to antibiotics when compared to similar anaerobic genera from the airways of healthy control subjects [33,34]. Therefore, this may indicate that anaerobic bacteria are established members

of the lower airway community and are undergoing adaptation to antibiotic selective pressure within the lungs.

Some strains of *P. aeruginosa* are associated with worse patient outcomes and similar patterns have been reported for periodontal microorganisms where differences in pathogenicity are observed between strains of the same species [35,36]. Therefore, further work is warranted to investigate genetic adaptations and diversity of particular anaerobic species in the CF lower airway environment.

Potential virulence mechanisms

Anaerobic bacteria are normal commensals but are also considered opportunistic pathogens, often involved in polymicrobial infections. Therefore, it is plausible that anaerobic bacteria could constitute members of the normal microbiota in health but also conceivable that they could contribute to the pathogenesis of chronic lung disease in people with CF via direct or indirect mechanisms. Recent evidence from a number of studies demonstrate that anaerobes may cause a host inflammatory response, interact synergistically with recognised CF pathogens and protect recognised pathogens from antibiotics by enhanced antimicrobial resistance (Figure 3).

Inflammation of the lower airways

Anaerobic bacteria in the CF lower airways may contribute to the dysregulated inflammatory response and subsequent, tissue destruction within the lungs [37**]. Bacteria including *Prevotella melaninogenica*, *Actinomyces odontolyticus*, *Veillonella parvula*, *Fusobacterium nucleatum* and

Streptococcus sanguinis secrete various short chain fatty acids (e.g. acetic acid, propionic acid, butyric acid) *in vitro,* which were also detected in micromolar amounts in bronchoalveolar lavage (BAL) fluid [37**]. Notably, high levels of acetic acid in BAL samples correlated with elevated levels of the pro-inflammatory cytokine interleukin-8 (IL-8), which is an important mediator of neutrophil recruitment and activation [37**]. The short-chain fatty acids were found to contribute to the excessive release of IL-8 by CF bronchial epithelial cells via a specific receptor (GPR41) that was overexpressed on the surface of the CF cells compared to non-CF bronchial epithelial cells [37**]. Another study also detected short-chain fatty acids in sputum of people with CF, which influenced host inflammatory responses and nitric oxide production and when present in low concentrations, improved the growth of *P. aeruginosa in vitro* [38**].

Interaction with other microorganisms in polymicrobial communities

Anaerobic bacteria may influence the progression of CF lower airway disease by inter-species interactions that enhance the pathogenicity or colonisation ability of recognised pathogens within the lower airways. In fact, it was previously hypothesised that certain anaerobes might create a favourable microenvironment for recognised CF pathogens to colonise, cause infection and subsequently dominate the lower airways (Figure 2) [27**].

It was also reported that co-infection of *P. aeruginosa* with *V. parvula*, in an *in vivo* murine model, resulted in the recovery of a significantly higher load of *P. aeruginosa* and greater clinical deterioration compared to infection with *P. aeruginosa* alone [39*]. Conversely, pyocyanin-producing *P. aeruginosa*

isolates may increase the virulence of obligate anaerobes. A study found that increased mortality was observed when mice were intra-tracheally inoculated with *Porphyromonas gingivalis* and a dose of pyocyanin at a concentration found in the CF airways compared to the anaerobe alone [40].

Biofilm formation is a feature of chronic airway infection in CF and it was shown that *P. aeruginosa* grows as a mixed biofilm *in vitro* with various *Streptococcus* species (e.g. viridans streptococci, *Streptococcus milleri* group) [41*,42]. Virulence factor production by *P. aeruginosa* was increased when grown as a mixed biofilm compared to without *Streptococcus* species [41*].

Furthermore, in patients with bronchiectasis it was reported that detection of non-tuberculous mycobacteria (NTM) in BAL samples was associated with a higher prevalence of *Prevotella* species compared to non-NTM infected patients [43]. Therefore, *Prevotella* species may affect colonisation of NTM in chronic lung infections.

Enhanced antibiotic resistance

A high proportion of CF-associated *Prevotella* species were previously shown to produce β-lactamases [33]. In polymicrobial infections, β-lactamase-producing bacteria may protect themselves and other members of the community from antimicrobial agents (the concept of “passive resistance”) [44]. A recent study found that a β-lactamase-producing *Prevotella* species was able to shield *P. aeruginosa* from the activity of ceftazidime *in vitro*, which is used to target the pathogen during treatment of pulmonary exacerbations [45*]. Therefore, enabling *P. aeruginosa* to persist in the presence of

ceftazidime may be an indirect method by which *Prevotella* species contribute to disease pathogenesis.

Should anaerobes be targeted by treatment?

There is currently a lack of evidence to conclude if anaerobic bacteria should be targeted when treating CF lower airway infection. Targeted treatment of bacteria, residing within the lungs, is also challenging with the types and formulations of antibiotics that are currently available. Anaerobic bacteria are generally susceptible to some broad-spectrum antibiotics that are routinely used in the management of CF lower airway infection (e.g. meropenem, piperacillin/tazobactam) [33]; therefore, it is likely that anaerobes are non-specifically targeted when recognised CF pathogens are treated. Furthermore, systemic antibiotics may adversely affect anaerobic bacteria that are present in high abundance in the normal microbiota of the gastrointestinal tract and contribute to disruption of the intestinal microbial community ("dysbiosis") [46]. CF is characterised by both local and systemic inflammation and evidence is accumulating that indicates a relationship exists between the gut microbiota and the health of the airways [47-50]. For example, disruption of gut homeostasis may result in immunoregulatory effects outside the intestinal tract, which may impact respiratory diseases including chronic obstructive pulmonary disease [51]. Therefore, any potential antibiotic treatment used to target anaerobes within the CF lower airways may need to be tailored to minimise adverse effects on the gut microbiota. Development of local therapies that specifically reduce bacterial interactions, limit the host inflammatory response triggered by anaerobes or minimise growth of target

anaerobes in the lungs, e.g. hyperbaric oxygen therapy, may offer an alternative [27**,37**].

Metagenomic sequencing studies generally indicate that in the absence of anaerobes, the health of patients is worse. Studies are required to understand the activities and interactions of microbes in early-stage disease and how the evolution of the bacterial community towards a less diverse end-stage disease (as found in explanted lungs) can be prevented [52]. We have also entered the era of CFTR modulators (e.g. correctors and potentiators), which target CFTR dysfunction, and how these treatments affect development of the airway community has been under short-term surveillance [28,53,54] but longer studies are required. Furthermore, the members and functional characteristics of the airway microbiota are individual to a person. Based on analysis of lung tissue samples from a symptomatic paediatric CF patient, who underwent a right lower lobectomy [55,56], bacterial communities are also known to be spatially heterogeneous in non-end stage disease. Therefore, virulence of anaerobic bacteria may differ both within and between people with CF.

In the future it may be possible to commence a personalised treatment approach that will maintain a normal microbial community, limit pathoadaptation of bacteria residing within the lower airways, prevent dominance by a recognised CF pathogen and subsequent lung damage in the individual. In fact, the Cystic Fibrosis Microbiome-determined Antibiotic Therapy Trial in Exacerbations: Results Stratified (CFMATTERS) trail is the first randomized, controlled trial comparing the use of microbiome-directed antibiotic treatment with standard therapy (tobramycin and ceftazidime) for

people with CF experiencing respiratory infections [57**]. In this clinical trial, which commenced recruitment in 2014, DNA is extracted from sputum and sequenced. Results are reviewed by a Consensus Expert Treatment Panel, who agree on a 3rd antibiotic treatment to add to standard therapy targeting *P. aeruginosa*. This 3rd antibiotic is selected to target additional bacteria detected in the airway microbiome.

Conclusion

Anaerobic bacteria are detected in respiratory samples provided by people with CF of all ages and stages of disease suggesting that they are common and persistent members of the lower airway community. The role of anaerobic bacteria in the CF lower airways might be as members of a normal airway microbiota with disruption of this microbiota and dominance of recognised pathogens responsible for disease progression. However, anaerobic bacteria also produce virulence factors and may contribute to a dysregulated inflammatory response or promote recognised CF pathogens to colonise and infect the lower airways. Additional studies are required to understand if specific anaerobic genera or species are important in health or disease of the CF airways and if this differs between individuals. Research investigating ways to prevent the evolution of the bacterial community towards a less diverse end-stage disease without disrupting the gut microbiota is commended.

(Word count: 2312)

Key points

- Anaerobic bacteria are commonly detected in the lower airways of people with CF but their clinical significance is unknown.
- The relative abundance of anaerobes in the lower airways fluctuates over time.
- A lower abundance of anaerobes has been associated with worse lung function suggesting that anaerobes may form part of a normal airway community.
- Anaerobic bacteria produce virulence factors and may enhance pathogenicity and colonisation by recognised CF pathogens.
- A personalised treatment approach might be required to prevent the development of a microbiota that is characteristic of end-stage lung disease.

Conflicts of interest

S.C.B. has served as a member of an Advisory Board to Vertex and Raptor, received travel support to attend and speak at symposia by Gilead and Novartis and acted as a lead investigator (site) of trials sponsored by Pharmaxis, Vertex and Raptor. M.M.T. is a co-investigator on the CFMATTERS trial and has served as a member of an Advisory Board to Bayer and received travel support to attend and speak at symposia by Novartis.

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This website provides details of the CFMATTERS study that is underway. This is the first randomised, controlled trial, which will use 'real-time' microbiome data to inform antibiotic treatment of a chronic infection.

Figure titles and legends

FIGURE 1. Prevalence of recognised respiratory pathogens among paediatrics and adults with cystic fibrosis (UK) in 2014 (n=9432) [3].

Abbreviation: MRSA, methicillin-resistant *Staphylococcus aureus*.

(Reproduced with permission from the UK Cystic Fibrosis Trust).

FIGURE 2. A potenial dynamic relationship between the relative abundance of anaerobic bacteria, recognised pathogens, lung function and inflammation in the cystic fibrosis airways over time.

(ORIGINAL)

FIGURE 3. Potential pathogenic mechanisms of anaerobic bacteria detected in the cystic fibrosis airways. Anaerobic bacteria may [A] increase the pathogenicity of recognised cystic fibrosis pathogens [B] produce short-chain fatty acids that exaggerate the host inflammatory response [C] shield recognised cystic fibrosis pathogens from treatment with antibiotics.

(ORIGINAL)

FIGURE 1

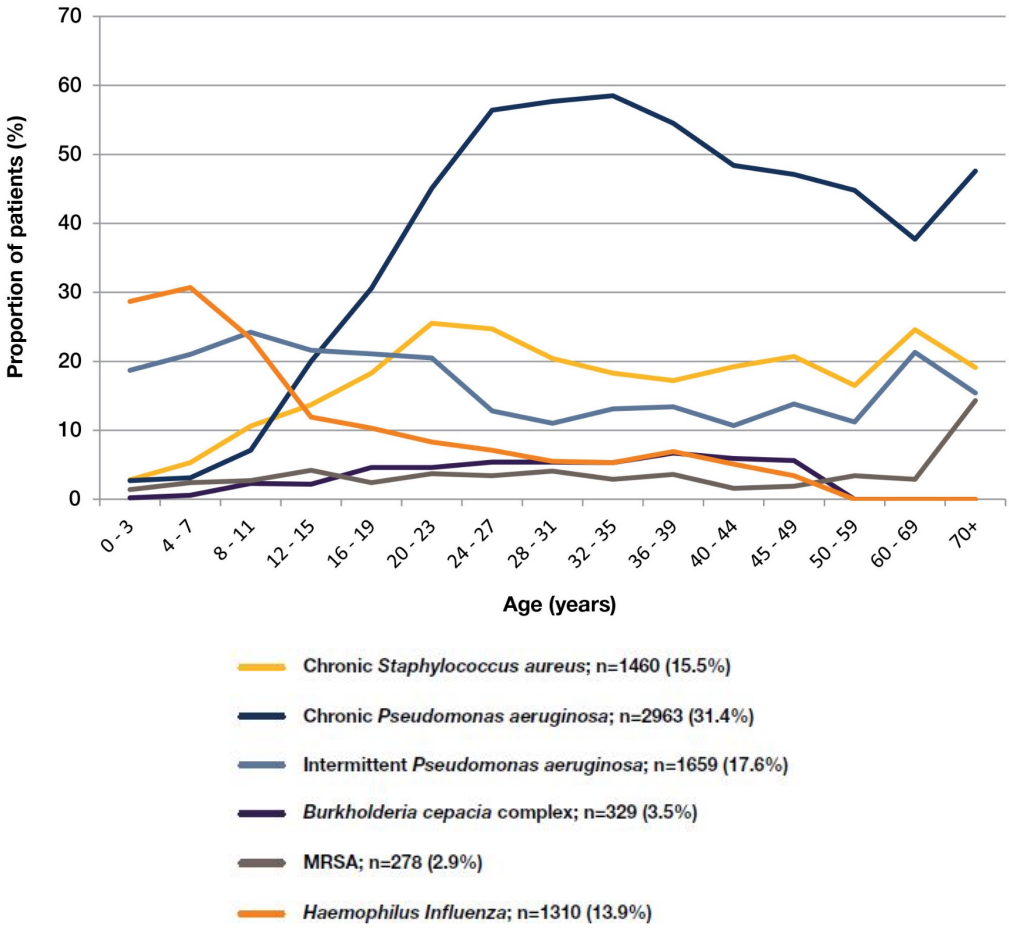

70
60
50
40
30
20
10
0
Proportion of patients (%)
0 - 3
4 - 7
8 - 11
12 - 15
16 - 19
20 - 23
24 - 27
28 - 31
32 - 35
36 - 39
40 - 44
45 - 49
50 - 59
60 - 69
70+
Age (years)
Chronic Staphylococcus aureus; n=1460 (15.5%)
Chronic Pseudomonas aeruginosa; n=2963 (31.4%)
Intermittent Pseudomonas aeruginosa; n=1659 (17.6%)
Burkholderia cepacia complex; n=329 (3.5%)
MRSA; n=278 (2.9%)
Haemophilus Influenza; n=1310 (13.9%)

FIGURE 2

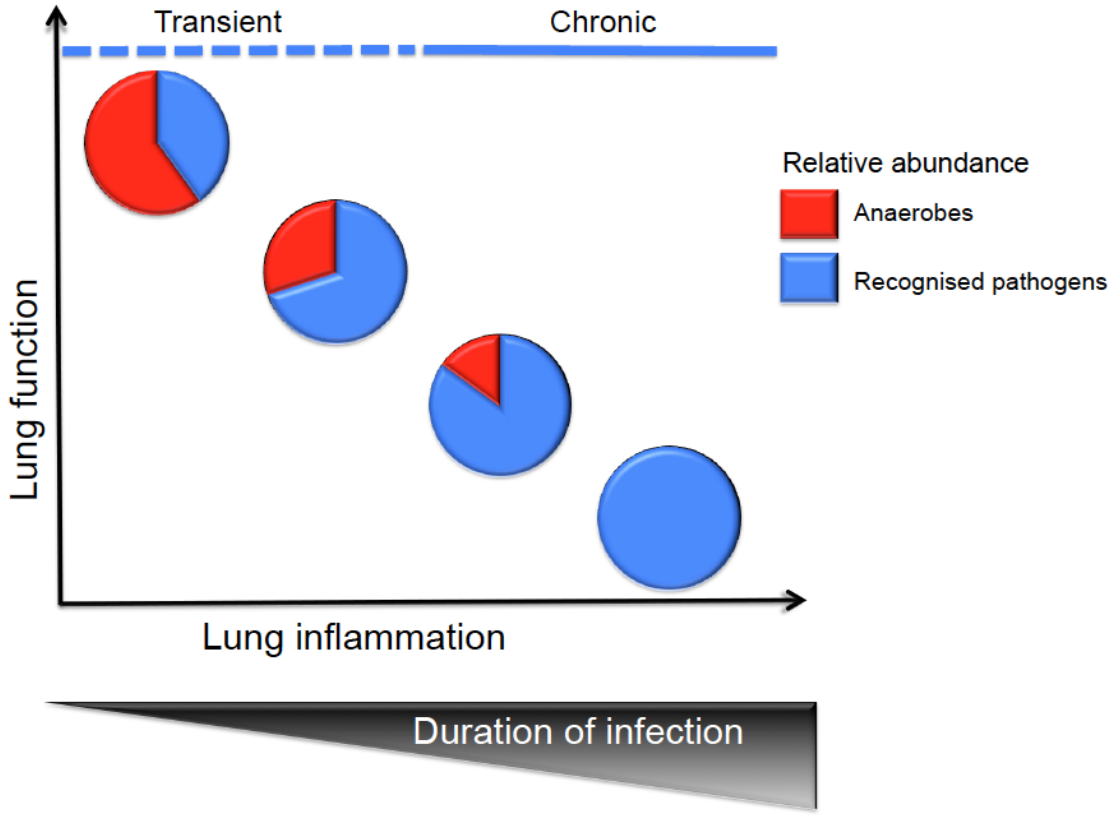
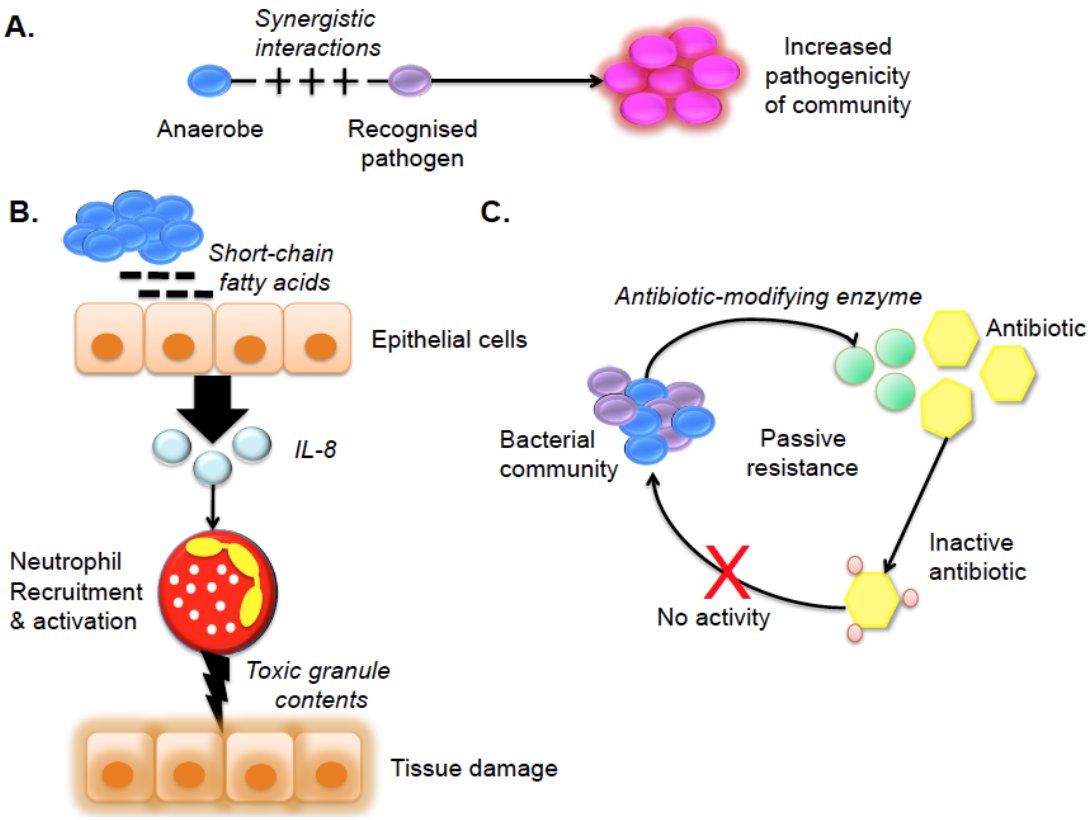

Transient
Chronic
Relative abundance
Anaerobes
Recognised pathogens
Lung function
Lung inflammation
Duration of infection

FIGURE 3


A.
Synergistic interactions
Anaerobe
Recognised pathogen
Increased pathogenicity of community
B.
Short-chain fatty acids
Epithelial cells
IL-8
Neutrophil Recruitment & activation
Toxic granule contents
Tissue damage
C.
Antibiotic-modifying enzyme
Antibiotic
Bacterial community
Passive resistance
Inactive antibiotic
No activity